Evaluation of the Yield of Bioethanol from Sterilized and Unsterilized Fruit Pulp of Waste Pineapple

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Abstract

In this research work, waste pineapple (*Ananas comosus* L.) was used as raw material for the production of bioethanol through direct fermentation by using baker's yeast (*Saccharomyces cerevisiae*). Both unsterilized and sterilized fruit pulp were fermented with baker's yeast (*Saccharomyces cerevisiae*) under anaerobic condition. Different fermentation conditions such as amount of substrate (100, 200, 300, 400, 500 and 600) g, amount of yeast (0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 % w/w), temperature of fermentation (28, 30, 32, 34, 36 and 38) °C, different pH (3.5, 4, 4.5, 5, 5.5 and 6) and period of fermentation (1, 2, 3, 4, 5 and 6) days were carried out to obtain the maximum strength of ethanol. The strength of the resultant bioethanol was measured by sp.gr and also analyzed by gas chromatography (GC). It was pointed out that the maximum strength of bioethanol 49.78 (%v/v) and 46.63 (%v/v) were obtained from sterilized and unsterilized pulp of pineapple respectively.

Keywords: Bioethanol, waste pineapple, sugary material, fermentation

Introduction

Global depletion of fossil fuels, rising fuel prices, environmental concerns, and pressures for oil independence are creating a strong market for biofuels. Biofuels have the potential to be domestically and globally available energy for energy security, with most being carbon neutral or potentially carbon negative and supportable within the current agricultural infrastructure (Shilpa, Malhotra, and Chanchal, 2013). Bioethanol is being widely explored as a renewable liquid fuel and widely accepted for clean burning. It is used as either an alternate fuel or blend with gasoline in many countries.

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Collection of suitable and economical source is considered for industrial ethanol production. Technically, ethanol can be produced from a wide variety of renewable feedstock, which can be roughly classified into three main groups: (1) those containing considerable amounts of readily fermentable sugars (sugar cane, sugar beets, and sweet sorghum), (2) starches (corn, potatoes, rice, wheat) and (3) celluloses (cornstover, grasses, corn cobs, wood, bagasse) (Walker, 2010).

Sugary materials such as sugar cane, sugar beet and sweet sorghum maintain the simple sugars such as sucrose, glucose and fructose that can be readily fermented by yeast. The cheapest and easily available source for the production of bioethanol is waste fruits (Ranganna, 1997). In the present research, ethanol was prepared from waste fruit pineapple (*Ananas comosus* L.) by fermentation. Fermentation of sterilized and unsterilized pulp was carried out with different amount of substrate, amount of yeast, temperature of fermentation, various pH and period of fermentation.

Materials and Methods

Materials

Waste pineapple (*Ananas comosus* L.) (ripe and overripe) produce of Thipaw Township, Shan State was collected from Thiri Mingalar Market, Hlaing Township, Yangon Region. Potassium sodium tartrate (analar grade), anhydrous Copper II sulphate (analar grade), sodium hydroxide (analar grade) and *Saccharomyces cerevisiae* (baker's yeast) were purchased from Kemiko,(Chemical Dealers), 28th Street, Pabedan Township, Yangon.

Methods

Fermentation Process

Waste pineapple was peeled and other spoiled parts were removed. Flesh of fruits were washed, sliced and pulped using a blender. Some pulp were sterilized in an autoclave at 121 ºC for 15 min. Some pulp were directly inoculated with baker's yeast (*Saccharomyces cerevisiae*) under anaerobic condition for fermentation. Fermentation of 500 g of sterilized pulp was conducted with 0.6 (%w/w) yeast for three days. Its pH was maintained at pH 4 and the temperature was kept at 32 °C. Fermentation of

unsterilized pineapple pulp was also carried out under the conditions as mentioned earlier for sterilized pulp. Yeast nutrients, 0.1 (%w/w) of diammonium phosphate were employed during fermentation of both sterilized and unsterilized pulp. Fermentation was maintained at different amount of substrate (100, 200, 300, 400, 500 and 600) g, amount of yeast (0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 % w/w), temperature of fermentation (28, 30, 32, 34, 36 and 38) °C, at different pH (3.5, 4, 4.5, 5, 5.5 and 6) and period of fermentation (1, 2, 3, 4, 5 and 6) days. Ethanol was then separated from the fermented broth by simple distillation at 78 ± 1 ℃. The resultant ethanol was measured using a density bottle at 20 ℃. The first distillate was further purified by fractional distillation at 78 ± 1 ℃ using fractionating column (50 cm in length, 3.2 cm in outside diameter, 2.5 cm inside diameter) packed with glass tubes (length = 1.5 cm, diameter = 0.25 cm). After 3 hr of fractionating time, the resultant second distillate was collected and analyzed by gas chromatography (GC).

Determination of Ethanol Content

The strength of ethanol for first distillate was measured by specific gravity method. Gas Chromatography (GC 2010, SHIMADZU) equipped with the flame ionization detector (FID) was then used for identification of second distillate at the Laboratory of Amtt Co. Ltd, Mayangone Township,Yangon Region.

Results and Discussion

In this research work, waste pineapple (*Ananas comosus* L.) was used as source of sugary material and converted into ethanol through fermentation by inoculating baker's yeast (*Saccharomyces cerevisiae*). During fermentation, effect of process variables such as amount of substrate, amount of yeast, temperature of fermentation, pH and period of fermentation was studied based on the maximum strength of bioethanol.

Effect of Process variables on the Strength of Bioethanol

Amount of Substrate and Amount of Yeast

Figure (1) shows the effect of the amount of substrate on the strength of ethanol for sterilized and unsterilized pulp. 500 g of substrate was the suitable amount for both sterilized and unsterilized pulp to obtain the maximum strength of ethanol 11.9 (%v/v) and 9.21 (%v/v) respectively. The amount of substrate was increased until 500g together with increased in the strength of ethanol. However, beyond 500g substrate, the strength of ethanol decreased. Yeast may be consumed sugary materials as source of carbon and microorganisms to food ratio may influence on its metabolic action to produce ethanol during fermentation process.

Figure (2) presents the effect of the amount of yeast on the strength of ethanol for sterilized and unsterilized pulp. The maximum strength of ethanol 12.84 (%v/v) and 9.84 (%v/v) for each pulp were obtained respectively using the amount of yeast 0.6 (%w/w). Even though the amount of yeast was increased above suitable amount of yeast, the strength of ethanol gradually decreased. This fact shown that the amount of yeast inoculated affected the yield of ethanol. It was also observed that higher ethanol content has resulted for sterilized pulp in which fermentation was based on substrate amount and carried out by using inoculated baker's yeast only. The strength of resultant ethanol from fermentation of unsterilized pulp was observed as low. Unsterilized pulp may contain backbone microorganisms apart from inoculated baker's yeast and the formation of other unwanted materials during fermentation caused the lower strength of ethanol.

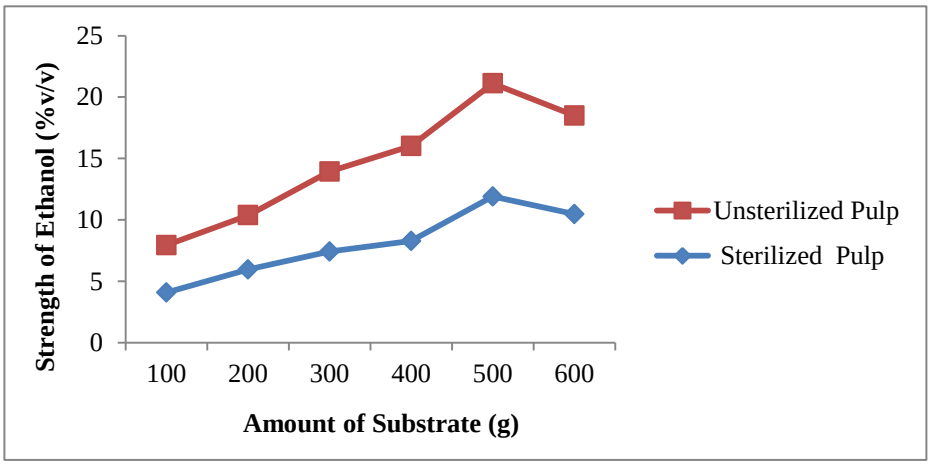

Figure 1. Effect of the Amount of Substrate on the Strength of Ethanol for Sterilized and Unsterilized Pulp

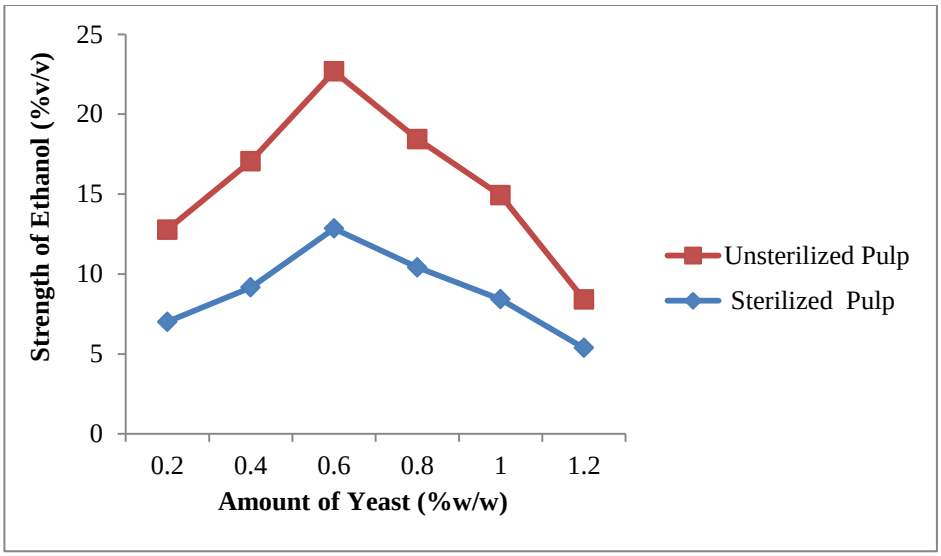

Figure 2. Effect of the Amount of Yeast on the Strength of Ethanol for Sterilized and Unsterilized Pulp

Fermentation Temperature

Fermentation Temperature is one of the most important factors that affects the ethanol production from sugary material as a carbon source using yeast. Ethanol production gradually increased during fermentation temperature of 28 to 32 °C and then sharply decreased with higher fermentation temperatures as can be seen in Figure (3). The maximum strength of ethanol 12.92 (%v/v) and 10.84 (%v/v) for both pulp have resulted at the fermentation temperature of 32 °C. The maximum yield of alcohol for both samples was obtained at that temperature. It was found that increasing the temperature above 32 °C could reduce the yield of ethanol. It may be mainly due to the denaturing of the yeast cells at elevated temperature.

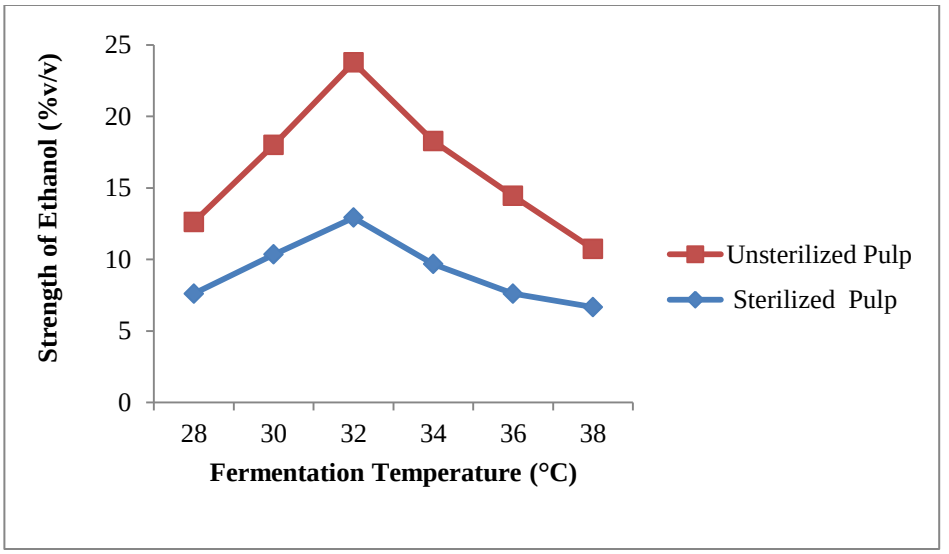

Figure 3. Effect of the Fermentation Temperature on the Strength of Ethanol for Sterilized and Unsterilized Pulp

pH

Figure (4) shows the effect of pH on the strength of ethanol for sterilized and unsterilized pulp. pH is an important environmental factor affecting cell growth and metabolite production. With increase in pH, yeast produces acid rather than alcohol. pH during fermentation should be controlled at low pH for two reasons: (1) the growth of other harmful microorganisms is retarded by acidic solution: (2) yeast grows well in acidic condition. Yeast survives in a slightly acidic environment with the pH between 4 to 6 (IJIRSET, 2013). From Figure (4), the maximum strength of ethanol 10.42 (%v/v) and 7.84 (%v/v) had been resulted for both pulp at the optimum pH 4.

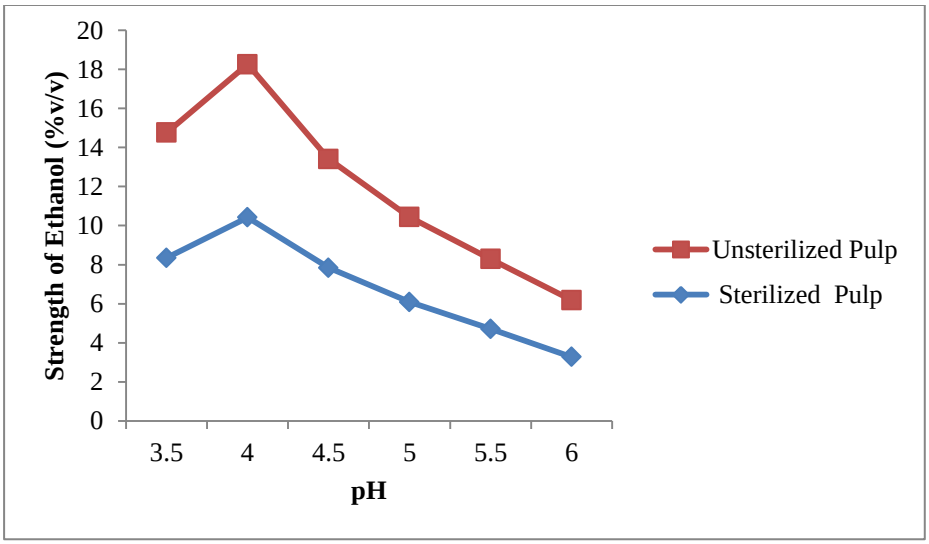

Figure 4. Effect of pH on the Strength of Ethanol for Sterilized and Unsterilized Pulp

Fermentation Period

Figure (5) shows the effect of Fermentation period on the strength of ethanol for sterilized and unsterilized pulp. The maximum strength of alcohol of 8.23 (%v/v) and 5.21 (%v/v) were obtained at the fermentation period of 3 day for both pulp respectively. It has been observed that the strength of ethanol did not increase even though the pulp were subjected to longer fermentation period. Longer fermentation period may offer some infection and unwanted microorganisms such as acetobacter and lactobacillus that could produce vinegar and lactic acid. In addition, a small amount of glycerol and lactic acid may be formed during fermentation process (Byarugaba-Bazirake, 2008). It may be depend on the structure and composition of fruits.

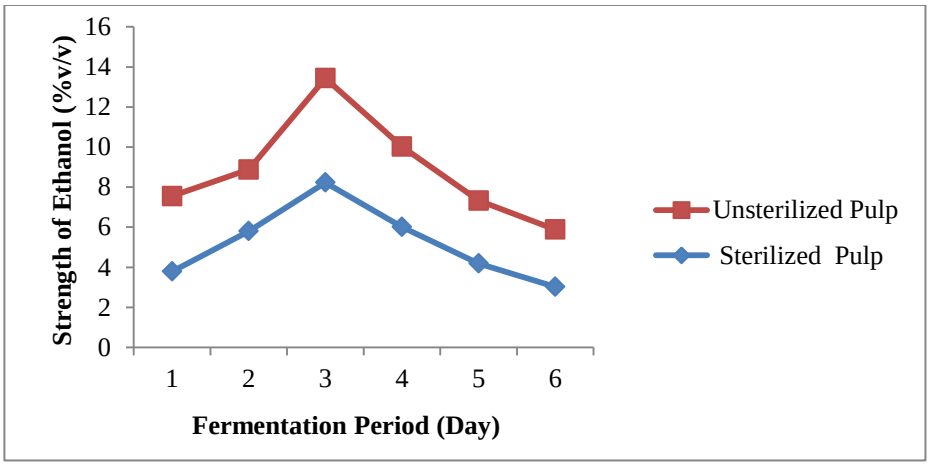

Figure 5. Effect of the Fermentation Period on the Strength of Ethanol for Sterilized and Unsterilized Pulp

Figure (6) shows the content of reducing sugar of both pulp was decreased from 57.10 mg/g to 0.12 mg/g and from 51.81 mg/g to 0.13 mg/g respectively on a 3- day fermentation period. It was found that baker's yeast used in this research was well adapted in the chosen conditions by consumption of sugar as sole carbon source.

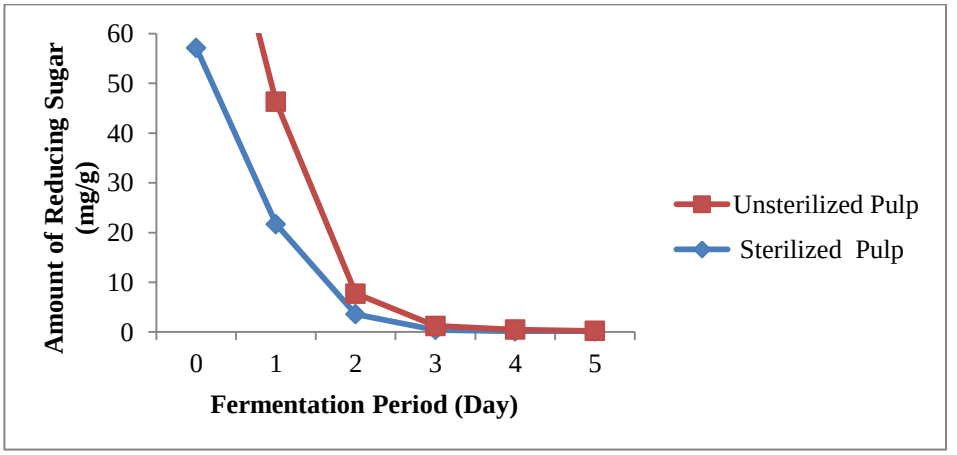

Figure 6. Amount of Reducing Sugar during Fermentation of Sterilized and Unsterilized Pulp

Table (1) presents the strength of ethanol analyzed by specific gravity was used to measure the strength of ethanol obtained from simple distillation. Sterilized pulp had the higher strength of ethanol (13.67 ±

0.35%v/v) than that of unsterilized pulp of (9.84 ± 1.00 %v/v). Gas chromatography (GC) was used to measure the strength of ethanol obtained from fractional distillation. Based on the GC analysis, sterilized pulp had the higher strength of ethanol 49.78 (%v/v) when compared to that of unsterilized pulp 46.63 (%v/v) as shown in Figure (7) (a) and (7) (b).

Table 1. Strength of Ethanol Analyzed by Specific Gravity and Gas Chromatography

Sr.No.	Sample	Strength of Ethanol (%v/v)	
		by sp.gr	by GC
1.	Sterilized Pulp of Pineapple	13.67 ± 0.35	49.78
2.	Unsterilized Pulp of Pineapple	9.84 ± 1.00	46.63

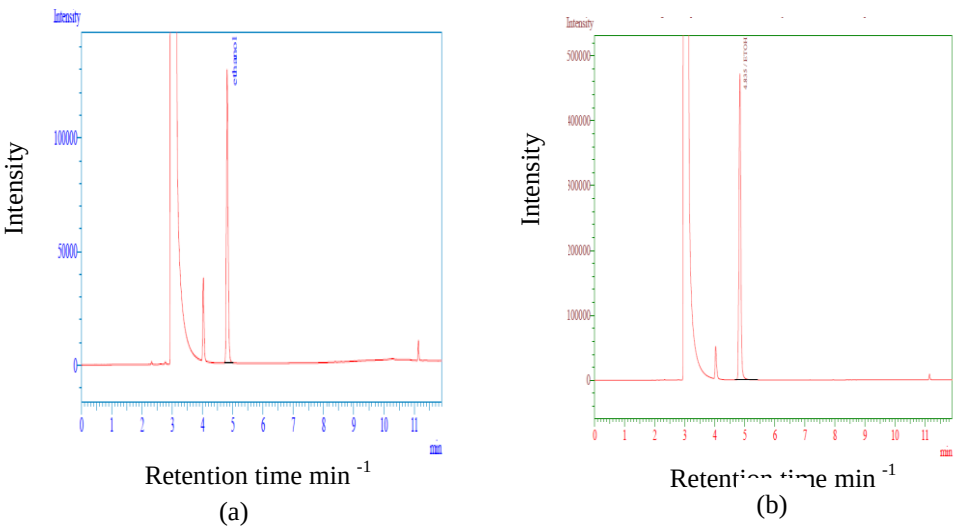

Figure 7. Gas Chromatography for Bioethanol from Sterilized and Unsterilized Pulp

Conclusion

In this study, waste pineapple (*Ananas comosus* L.) was used as raw materials for the production of bioethanol through direct fermentation by using baker's yeast (*Saccharomyces cerevisiae*). Based on the analysis of sp.gr and GC, the strength of ethanol obtained from sterilized pulp had higher than that of unsterilized pulp. However, the strength of ethanol from sterilized pulp was not significantly higher than that of unsterilized pulp. In fermentation, sterilized pulp was used as raw materials for the production of ethanol, pure ethanol could be produced. If unsterilized pulp was used, not only ethanol but also undesirable products could be produced. Moreover, the volume of resultant ethanol from the fermentation process may not the same for sterilized and unsterilized pulp.

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